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## **Behavioural type in newly emerged steelhead *Oncorhynchus mykiss* does not predict growth rate in a conventional hatchery rearing environment**

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Behavioural assays were conducted on newly emerged steelhead *Oncorhynchus mykiss* to investigate the presence of behavioural syndromes and to determine whether behavioural type in young fish predicts growth rate in a conventional hatchery rearing environment. Individual fry were consistent in their position choice and activity behaviours across safe and unsafe contexts, as well as among assays conducted on different days. Position choice and activity behaviours, however, were not necessarily correlated to each other. Both behaviours predicted feeding rates during behavioural assays, but there was no relationship between fry behaviour and subsequent growth rate or survival during the first 3 months of hatchery rearing. These results support the hypothesis that selection in captivity may be relaxed with respect to behavioural type rather than directional, allowing for increased behavioural variance in domesticated populations. Modest magnitudes of correlations among fry behaviours, however, suggest that behavioural type may be unstable at the onset of the juvenile feeding stage.

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**Key words:** activity; behavioural syndromes; boldness; domestication.

### **INTRODUCTION**

Behavioural syndromes, or suites of behavioural traits that co-vary across multiple contexts or situations, are becoming accepted as a widespread phenomenon among animal taxa (Gosling, 2001; Sih *et al.*, 2004a; Bell, 2007; Réale *et al.*, 2007). A growing number of studies have documented individual behavioural consistency in fish taxa, particularly with respect to aggression (Francis, 1990; Budaev *et al.*, 1999) and risk-taking behaviour or boldness (Murphy & Pitcher, 1991; Coleman & Wilson, 1998; Magnhagen & Staffan, 2005). Thus, within a boldness syndrome in a population, individuals express a consistent behavioural type such that they maintain a steady rank-order of boldness levels across multiple situations. In a number of examples, boldness levels are also positively associated with aggression (Huntingford, 1976; Bell & Stamps, 2004; Sundström *et al.*, 2004; Bell, 2005; Bell & Sih, 2007). Other work has shown that boldness levels are also linked with fitness-related

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traits (Smith & Blumstein, 2008), such as body size (Brown & Braithwaite, 2004; Brown *et al.*, 2005), condition (Brown & Shine, 2007) and feeding performance (Staffan *et al.*, 2005). These relationships suggest important ecological and evolutionary implications of behavioural syndromes, such as constraints on behavioural plasticity required for achieving optimal behaviour in all life contexts (Dall *et al.*, 2004; Sih *et al.*, 2004b; Smith & Blumstein, 2008).

Recent research in salmonids has also adopted a behavioural syndromes framework. Evidence for behavioural syndromes in salmonids includes consistent individual differences in boldness in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Sneddon, 2003; Wilson & Stevens, 2005), which are linked to other traits such as acclimation time to unfamiliar environments (Øverli *et al.*, 2002), stress coping style (Øverli *et al.*, 2006) and learning ability (Sneddon, 2003). In brown trout *Salmo trutta* L., recent studies suggest that boldness traits in juveniles are sex specific (Johnsson *et al.*, 2001) and linked with aggression (Sundström *et al.*, 2004).

To date, the majority of the behavioural syndrome research in salmonids has focused on the advanced parr and yearling life stages (Sneddon, 2003; Wilson & Stevens, 2005; Salonen & Peuhkuri, 2006; Laakkonen & Hirvonen, 2007; Sundström *et al.*, 2004), despite the evidence from Atlantic salmon *Salmo salar* L. that behaviour expressed during initial feeding stages may be important to growth trajectory and life-history strategies. In this system, fry that are first to emerge from redd gravel are dominant over fish emerging later, even when effects of prior residence and body size are controlled (Metcalf & Thorpe, 1992). These early-emerging fry have higher standard metabolic rates (Metcalf *et al.*, 1995), higher social rankings and faster growth rates during freshwater residency and, as a result, they achieve body size thresholds for smoltification sooner than late-emerging fry (Metcalf & Thorpe, 1992; Metcalf, 1998). While this work in *S. salar* has demonstrated important links between behavioural traits early in life and overall life history, little is known with respect to correlations among behavioural traits or behavioural consistency in young fry (*i.e.* behavioural syndromes), even though boldness and aggression syndromes are apparent among parr and yearlings (Sneddon, 2003; Wilson & Stevens, 2005; Øverli *et al.*, 2006).

Beyond an improved understanding of the presence of a known syndrome in different life stages of salmonids, investigations of behavioural syndromes in young salmonids are also pertinent to conservation efforts that use artificial propagation for population enhancement and reintroduction programmes. Salmonids are an intensively farmed group of fishes, and the use of captive-reared fishes for population enhancement efforts is increasingly common as many wild salmonid populations have declined (Flagg & Nash, 1999). Recent work suggests that reintroduction programmes may benefit from pre-release efforts that foster a diversity of behavioural types (Watters *et al.*, 2003; Watters & Meehan, 2007). The rationale behind this argument is that consistent individual behavioural variation may represent alternative behavioural strategies that lend resilience to the population in an unpredictable environment, thereby improving the chances that the population will sustain itself after release.

Numerous studies have demonstrated differences in mean behavioural phenotype between hatchery-origin and wild salmonids and generally agree that hatchery-origin salmonids are bolder and more aggressive than their wild counterparts (Huntingford, 2004; Huntingford & Adams, 2005). Collectively, these findings suggest that

salmonid hatcheries exert directional selection for individuals of an aggressive and bold behavioural type, such that there is reduced variation in hatchery populations with respect to these traits. Since most of the work examining differences between hatchery and wild stocks to date has compared average behaviour between the two groups and has not tracked the performance of individuals expressing different behavioural types in the hatchery environment, this hypothesis has not been directly tested. Nonetheless, if true, the directional selection hypothesis predicts overall low variation in established hatchery populations, and where individual behavioural variation is observed, relatively bold and aggressive individuals should be more successful (e.g. more likely to grow and survive) than shy, subordinate fishes in a conventional hatchery environment.

An alternative hypothesis proposes that selection in the captive environment is relaxed rather than directional. In this vein, McPhee (2004) found that behavioural variance in oldfield mice *Peromyscus polionotus* increased over generations in captivity, presumably due to a lack of selection pressure against maladaptive behaviour. In support of the notion that the simple environments in common in captive rearing situations may encourage increased behavioural variance, Lee & Berejikian (2008a) observed increased variability in exploratory and feeding behaviours among juvenile steelhead *O. mykiss* raised in a barren, unstructured environment compared with those raised in environments with either stable structure or structurally variable environments. Contrary to the directional selection hypothesis, the relaxed selection hypothesis predicts a high degree of individual variation among hatchery-raised fishes and no relationship between behavioural type and growth or survival in the hatchery environment.

By investigating individual consistency in behaviour and behavioural correlations at the beginning of the juvenile feeding stage, the presence or absence of a behavioural syndrome known to exist in older fishes, as well as the degree of individual variation, can be established prior to a significant period of hatchery selection. The alternative hypotheses of directional or relaxed domestication selection on behaviour can then be tested by examining the performance of individuals in a conventional hatchery setting with respect to their behavioural type.

The primary objectives of this study were to investigate the presence of behavioural syndromes in young *O. mykiss* fry and, if individual behavioural types could be established, measure the relative performance of those behavioural types within a conventional hatchery rearing system. To examine behavioural syndromes at an early age, a series of three behavioural assays were carried out to assess individual consistency and variation in boldness and its relationship with aggressive behaviour among newly emerged fry within an established hatchery population of *O. mykiss*. Two assays assessed individual response to two stressful events, handling stress and a simulated predator attack. A third trial evaluated aggression *via* the response of focal individuals to a conspecific introduced into observation aquaria. To investigate selection for boldness and aggressiveness in the hatchery environment, individual survival and growth rates of the observed fry were followed over the first 2.5 to 3.0 months of rearing. Growth rate was used as the primary indicator of success because it is a common measure of performance in aquacultural systems (Bryden *et al.*, 2004). In addition, body size of hatchery-raised salmonids is a correlate of post-release survival (Lundqvist *et al.*, 1994), and therefore may be an indicator of fitness after hatchery rearing.

## MATERIALS AND METHODS

All fry were sampled for observation in the spring of 2005 from the Don Clausen–Warm Springs Fish Hatchery (WSH), located in Geyserville, California. This hatchery has operated a mitigation programme for *O. mykiss* since completion of the Warm Springs Dam in 1981 on Dry Creek, a major tributary to the Russian River. In a typical cycle, *O. mykiss* are raised at WSH until they are released as yearlings to complete the ocean phase of their life cycle and return to the hatchery to be spawned as adults. Natural origin returns to the hatchery are not incorporated into the population. Thus, the subjects for this study were from the eighth generation of the hatchery programme, assuming an average generation time of 3 years (Moyle, 2002). Adult returns to WSH are sampled for spawning on a weekly basis over the course of the run, with up to eight females and the same number of males used for broodstock on each spawn date.

## BEHAVIOURAL ASSAYS

Sixteen fry were randomly sampled for individual observation from the progeny of eight consecutive spawn dates, such that eight sets of behavioural assays, or study blocks, were carried out over 8 weeks. Two fry died during the assays and were excluded from analysis, for a final sample size of 126 individuals for the fry behavioural data. All fry were sampled at random from general hatchery rearing troughs between 1600 and 1800 hours, 5–13 days after their emergence and placed in individual aquaria (40 × 20 × 25 cm, water depth of 14 cm) for observation. As in most conventional hatcheries, *O. mykiss* eggs and alevins were housed in large tanks containing tens of thousands of individuals, and individual fry from a single spawn date emerged over *c.* 7 days. Therefore, it was not possible to ascertain the exact number of days since emergence for each individual subject. The age range of the observed fry reported here gives the maximum possible ages of individual fry across all study blocks (number of days at sampling since the first fry emerged in the tank sampled).

There was no water flow through the observation tanks; instead, they were arranged in two rows of eight aquaria inside two shallow troughs (125 × 30 × 20 cm) with flowing water, such that constant water flow in the outer troughs maintained water temperature at 10.5–11.5° C within the aquaria. Airstones placed in each tank maintained dissolved oxygen levels at 7.0–9.0 mg l<sup>-1</sup>. Aquaria were not cleaned between behavioural assays to avoid unnecessary disturbance. The ends of each tank were covered with black plastic to keep the subjects in visual isolation of each other. Each tank was also equipped with a rotary feeder that could be operated remotely by pulling a cord to release food into the tank.

The three behavioural assays consisted of two 5 min observations of position choice, activity and feeding performance, performed before and after three different stimuli: (1) a handling stress event in which individuals were netted with an aquarium net and held out of the water in the net for 20 s; (2) a simulated predator attack, in which a model heron skull was used to strike at the focal individual three times in rapid succession; (3) introduction of a conspecific placed within a 50 ml glass volumetric flask. For all assays, the first observation was performed 30 min before the stimulus presentation and the second observation took place directly after presentation of each stimulus. Activity was recorded as the total number of lines crossed over a 30 × 30 mm grid marked on the bottom of each aquarium. Position was recorded every 30 s as either open or not open, resulting in 10 point observations for each 5 min observation period. Open positions were considered central tank areas, away (> 1 body length) from edges or shadowed areas. Not open positions included the use of the airstone as shelter, or tank edge positions, where fry were camouflaged by the black moulding that covered 1.2 cm of the tank perimeter. At the beginning of each observation, a measured amount of feed (0.05 g of starter feed, BioOregon, Inc; [www.bio-oregon.com](http://www.bio-oregon.com)) was released with the remotely operated feeder, and the total number of feedings was recorded. All observations were performed between 1600 and 2030 hours behind observation blinds outfitted with viewing window. Fry were fed *ad libitum* between assays *via* the automatically operated rotary feeders.

In preparation for the simulated predator attack, a PVC stand holding the model heron skull was placed over the tank 10 min prior to the attack, such that the skull hovered *c.* 0.5 m

above the tank. The model skull was then lowered from behind a blind *via* a string attached to the skull such that the observer did not have to approach the tank at any point during the procedure. Previous studies have used similar methods to elicit antipredator behaviour in juvenile salmonids and other fishes (Giles & Huntingford, 1984; Johnsson *et al.*, 2001; Bell & Sih, 2007).

For the response to the conspecific assay, the volumetric flask, filled only with water, was introduced to the aquarium at the start of the first 5 min observation of position and activity, thus allowing a 30 min adjustment period to the flask. Afterwards, the empty flask was replaced with an identical flask containing a conspecific of the same size as the focal individual. The second observation began immediately, during which the number of approaches to the conspecific was observed, in addition to position choice and activity. A movement toward the flask was only counted as an approach if it ended within one body length or touching the flask, in the portion of the flask containing the conspecific. To avoid distracting the focal individual from the conspecific, feeding performance was not observed before or after introducing the conspecific.

The first assay took place 24 h after transfer to observation aquaria, followed by the second and third assays at 24 to 28 h intervals. To address the possible effect of assay sequence on behavioural responses, the sequence was varied for study blocks 2 to 8. As there were six possible sequences for the three assays, two sets of three sequences were alternated between study blocks (for a range of 16 to 38 individuals per assay sequence).

## GROWTH DATA

At 24 h after the last behavioural assay, mass ( $M$ ) and fork length ( $L_F$ ) were measured (mean  $\pm$  s.d.  $L_F$ :  $28 \pm 2$  mm, mean  $\pm$  s.d.  $M$ :  $0.21 \pm 0.05$  g), and all fry were individually marked with subcutaneous visible implant elastomer (VIE) tags (Northwest Marine Technologies, Inc.; [www.nmt-inc.com](http://www.nmt-inc.com)). Different combinations of tag location and colour were used to achieve individual marks within long-term rearing tanks. Since it was necessary to add additional fry to the group rearing tanks to increase rearing densities to hatchery norms, the adipose fins of all observation subjects were clipped off to provide a batch mark for study subjects in case their individual VIE tags were lost.

After marking, fry were transferred to one of two rearing troughs ( $4.8 \text{ m} \times 1 \text{ m}$ , water depth maintained at 0.2 m for first 2 weeks of rearing, then raised to 0.4 m afterwards, in accordance with normal hatchery rearing practices for young fry), which were divided into two equal sections (upper and lower), resulting in four rearing units. Fry from two study blocks (32 individuals) were combined in one rearing section with 170 unmarked fry from the same age group. These tanks were located outdoors and therefore were exposed to a natural photoperiod ( $38^\circ 43' \text{ N}$ ). Individual body size measurements were collected 28 to 38 days after the initial fry marking (mean  $\pm$  s.d.  $L_F$ :  $48 \pm 5$  mm; mean  $\pm$  s.d.  $M$ :  $1.34 \pm 0.42$  g) and again 72 to 89 days after the initial fry marking (mean  $\pm$  s.d.  $L_F$ :  $72 \pm 8$  mm; mean  $\pm$  s.d.  $M$ :  $4.32 \pm 0.15$  g). Following standard feeding protocols at WSH, each tank section was fed daily a diet of BioOregon semi-moist starter pellets at a rate of 3% of the total section biomass for the first 4 weeks, after which the rate was reduced to 2.5% of total section biomass for the remainder of the rearing period. Portions of the daily ration for each rearing section were broadcast by hand three to five times each day. This feeding schedule was equivalent to feeding the population *ad libitum*, as excess food was observed at the bottom of the tanks after each day of feeding. In keeping with standard WSH rearing protocol, the average body mass of individual fish was estimated every 2 weeks by using an aquarium net to sample fish at random into a bucket with as little water as possible. The total mass of the fish placed in the bucket was then measured (generally 500–1000 g) and the number of fish in the bucket was counted to obtain the average body mass per fish. The procedure was repeated three times for each rearing unit and the approximation of average mass per fish was used to update feed rations.

During growth data collection at the 28–38 day interval for the first study blocks, it was apparent that some VIE tags had become impossible to see. To halt continued tag loss, all individuals from study blocks 5 to 8 were re-tagged 7–15 days after the initial tagging and re-tagged again at the 28 to 38 day data collection point. Tag loss prior to this re-tagging



procedure caused a reduction in sample size to 98 for the 28 to 38 day body size data collection, and finally to 90 individuals at the 72 to 89 day data collection.

## STATISTICAL ANALYSES

All behavioural and growth data were checked for normality with Shapiro–Wilk tests prior to analysis. As none of the behavioural measurements were normally distributed ( $P < 0.05$  for all tests), non-parametric tests were used when analysing behavioural data. For all analyses, position choice for each 5 min observation period was summarized as the proportion of the 10 point observations that a fish was in an open position, and these proportions were arc sine transformed before analysis. To test whether prior experience influenced activity, position, feeding or aggression, the response to each stimulus was tested for effects of assay sequence with Kruskal–Wallis tests. If the overall test indicated significant effects of sequence, response means were compared for individual sequence pairs with Wilcoxon rank-sum tests and the procedure for controlling false discovery rate (FDR) was followed (Benjamini & Hochberg, 1995) before declaring significant differences between any pair of sequences. Behavioural data were also checked for tank effects with Kruskal–Wallis tests.

To examine effects of exposure to a stressor or a competitor on mean behaviour, average activity, position and feeding were compared before and after the assay event with Wilcoxon rank-sum tests. A chief goal during analysis, however, was to assess individual variation and consistency in behaviour. Since activity and position choice have previously been identified as behavioural correlates of boldness in older *O. mykiss* subjects (Sneddon, 2003), these measurements were used to assess individual consistency in behaviour. In contrast, feeding rate was interpreted as an indicator of performance, rather than behavioural type. To examine individual consistency in activity and position choice across safe (pre-stimulus) and unsafe (post-stimulus presentation) conditions and over time, Spearman's coefficients of rank correlation ( $r_s$ ) were calculated for each behaviour both across conditions and across assays conducted on different days. To evaluate correlation matrices for both behaviours, Bartlett's test of sphericity was used to determine whether the matrices differed from the identity matrix (Bartlett, 1950). To assess significance of  $P$ -values for individual  $r_s$  values, the procedure for controlling FDR was used (Benjamini & Hochberg, 1995). Control of FDR has been recommended as an alternative to the highly conservative sequential Bonferroni procedure (Rice, 1989) because it offers increased power for detecting significant effects while still offering a correction for inflated type I error rates when carrying out multiple tests or comparisons (García, 2003, 2004).

Beyond individual consistency in any single behaviour, behavioural syndromes are often composed of multiple correlated behaviours (Huntingford, 1976; Sundström *et al.*, 2004; Bell, 2005; Salonen & Peuhkuri, 2006; Bell & Sih, 2007). To thoroughly assess the influence of a behavioural syndrome, a principal component analysis (PCA) was conducted using all activity and position choice variables. Calculation of the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy confirmed that the full correlation matrix including both position choice and activity observations was acceptable for use in a PCA (KMO = 0.703) (Kaiser, 1970; Dziuban & Shirkey, 1974). The number of components to interpret from the PCA was determined *via* three methods: (1) scree test (Cattell, 1966), (2) a parallel analysis (Zwick & Velicer, 1986) and (3) bootstrap resampling of the PCA and subsequent evaluation of the bootstrapped eigenvalues and eigenvector coefficients. The PCA was bootstrapped 100 times and, as described in Jackson (1993), the 95% CI of both the bootstrapped eigenvalues and the eigenvector coefficients were evaluated to determine the interpretability of each PCA component. The combination of these three methods of assessing component significance indicated the extraction of the first two components of the original PCA. The Varimax rotation was then applied to these two components for interpretation of loading values for individual variables. Loading values with absolute values of at least 0.30 were considered meaningful for component interpretation (Bryant & Yarnold, 1995). To collapse the multiple measures of both position choice and activity into single measures for the purpose of further analysis, scores from the two rotated components were then used as indices of fry behaviour.

PCA-derived behavioural indices were first used to examine the influence of fry body size on behaviour by calculating  $r_s$  between each score and ln-transformed  $L_F$  and  $M$ . The mean

number of feedings over all observations (pre and post-handling stress and simulated predator attack) was used as an index of feeding performance. To determine whether behaviour predicted feeding performance, a multiple regression was performed, using both behavioural indices as model effects and body size (ln-transformed  $L_F$ ) as a covariate. To relate activity and position choice indices with aggression, fry that exhibited some level of aggression by approaching the conspecific at least once were categorized as responders, while fry that did not approach the introduced conspecific at all were termed non-responders. Behavioural indices were compared between responders and non-responders with a nominal logistic regression model, again using ln-transformed  $L_F$  as a covariate.

The subjects varied in body size at the beginning of the first growth period ( $L_F$  range: 22–34 mm;  $M$  range: 0.11–0.39 g). It is well documented that growth rate in fishes varies as a function of body size (Ricker, 1979). To account for variability in growth rate due to variation in initial body size, relative growth rate in mass  $G_M$  was calculated as follows:  $G_M = (M_2 - M_1)(M_1 D)^{-1}$ , where  $M_2$  is the final body mass,  $M_1$  is the initial body mass and  $D$  is the growth interval in days. Relative growth rate was also calculated with  $L_F$  data ( $G_L$ ), from  $G_L = (L_{F2} - L_{F1})(L_{F1} D)^{-1}$ . Relative growth rates for each interval were analysed for effects of tank, rearing section (upper or lower portions of the tank) and study block with a three-level nested ANOVA, in which study block was nested within rearing section, and rearing section was nested within tank. To test the relationship between fry behaviour and subsequent  $G$ , multiple regressions for each growth period were carried out with PCA-derived behavioural indices as model effects and  $G$  as the response variables.  $G$  was also compared between conspecific responders and non-responders with a  $t$ -test. Mortalities occurring during the post-observation growth period were recorded, and their behavioural indices were compared with those of survivors with  $t$ -tests.

## RESULTS

### BEHAVIOURAL ASSAYS

Fry activity, time in the open and number of feedings were all significantly reduced after the simulated predator attack (activity and position choice distributions; Figs 1 and 2) and after handling stress (Wilcoxon rank sums, d.f. = 1 for all tests; handling stress activity,  $P < 0.001$ ; handling stress time in open,  $P < 0.05$ ; handling stress feedings,  $P < 0.001$ ; predator attack activity,  $P < 0.001$ ; predator attack time in open,  $P < 0.05$ ; predator attack feedings,  $P < 0.001$ ). There was no difference, however, in activity or time spent in open areas before *v.* after introducing a conspecific (Wilcoxon rank sums, d.f. = 1,  $P > 0.05$  for both tests). Responses to each assay event were not affected by assay sequence (Kruskal–Wallis tests, d.f. = 5,  $P > 0.05$  for all tests). There was also no effect of observation tank on activity, position choice, or feeding, before or after assay events (Kruskal–Wallis tests, d.f. = 15,  $P > 0.05$  for all tests).

Although fry activity and time spent in open areas was reduced after the predator attack and handling stress, correlation matrices for activity rates and position choice across all assays revealed individual consistency in rank levels of behaviour across safe and unsafe conditions. That is, fry that were most active or spent the most time in exposed positions prior to the stimuli, continued to be relatively active or were more likely to choose open positions after the stressful event (Tables I and II). Activity and position choice were also significantly correlated before *v.* after introduction of a conspecific (Tables I and II). When activity was compared between observations conducted on different days, correlations were significant in some, but not all, cases. For example, activity was significantly correlated across

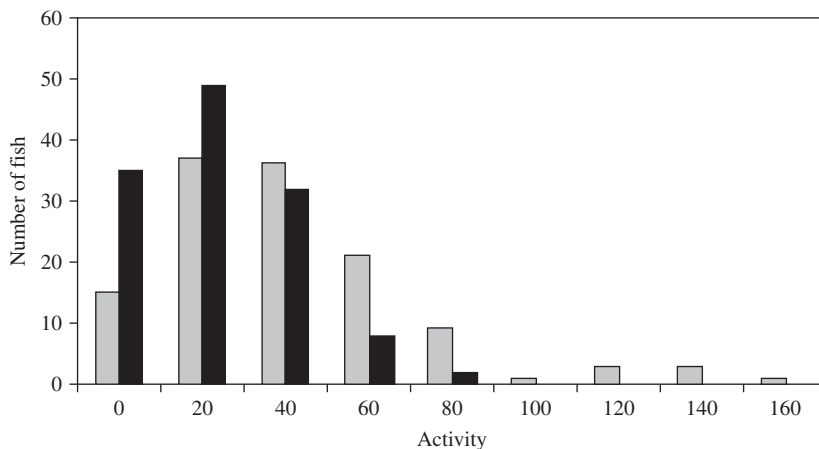


FIG. 1. Activity distributions of *Oncorhynchus mykiss* fry before (□) and after (■) the simulated predator attack.

two of three possible combinations of pre-stimulus contexts. Additionally, activity in post-stimulus conditions was correlated only between the predator attack and response to conspecific trials (Table I).

Between assays conducted on different days, fry were more consistent in their position choice than they were in their activity levels. In pre-stimulus conditions, all correlations were significant, and after handling stress, time spent in the open was significantly correlated with position choice after the predator attack and introduction of a conspecific (Table II). Only a single coefficient in the position choice matrix (pre-predator *v.* post-conspecific) was not statistically significant (Table II). Notably, nearly all correlation coefficients for both activity and position choice matrices were

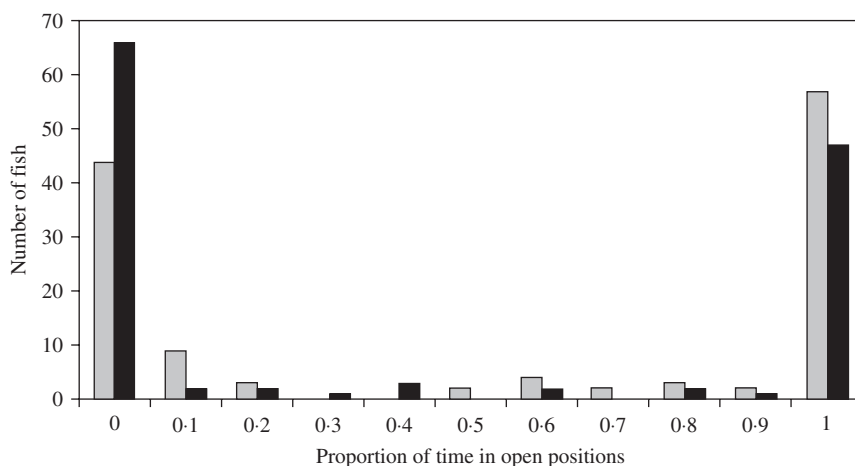


FIG. 2. Distributions of the time spent by *Oncorhynchus mykiss* fry in open position before (□) and after (■) the simulated predator attack.



TABLE I. Spearman's coefficients of rank correlation ( $r_s$ ) between *Oncorhynchus mykiss* fry activity levels before and after stimulus presentations within assays and across assays conducted on different days ( $n = 126$  for all correlations)

	Pre-predator	Pre-conspecific	Post-handling	Post-predator	Post-conspecific
Pre-handling	0.27**	0.16	0.30***	0.27**	0.21*
Pre-predator	—	0.32***	0.13	0.36***	0.14
Pre-conspecific	—	—	-0.02	0.17	0.38***
Post-handling	—	—	—	0.12	0.11
Post-predator	—	—	—	—	0.21*

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

Raw probability levels are given; however, all noted coefficients remained significant after following the procedure for controlling false discovery rate.

positive and of generally modest magnitude, with a majority of values registering as statistically significant. Bartlett's tests of sphericity indicated that both matrices differed significantly from the identity matrix ( $P < 0.001$  for both matrices).

The first two components from the PCA on both behaviours were retained for further analysis (37% of the total variance explained; Table III). Loading values for the first component (PC-Open, accounting for 25% of total variance) were interpretable for all observations of the proportion of time fry spent in open positions as well as activity prior to the simulated predator attack. For the second component (PC-activity, accounting for 12% of the total variance), five of six activity variables were interpretable, as well as time spent in open positions before and after handling stress (Table III). All meaningful loading values were positive for both components. While the two behaviours were generally segregated between the two components, positive loadings for one activity variable on the PC-open component and two position choice variables on the PC-activity component probably reflect modest correlations between the two behaviours. While the full correlation matrix between activity and time in open positions was not used for formal analysis of a behavioural syndrome, a cursory examination of the 36 possible correlation coefficients revealed weakly

TABLE II. Spearman's coefficients of rank correlation ( $r_s$ ) between time spent by *Oncorhynchus mykiss* fry in open positions before and after stimulus presentations within assays and across assays conducted on different days ( $n = 126$  for all correlations)

	Pre-predator	Pre-conspecific	Post-handling	Post-predator	Post-conspecific
Pre-handling	0.39**	0.29**	0.39**	0.38**	0.19*
Pre-predator	—	0.34**	0.19*	0.46**	0.06
Pre-conspecific	—	—	0.21*	0.19*	0.38**
Post-handling	—	—	—	0.30**	0.27**
Post-predator	—	—	—	—	0.22*

\*  $P < 0.05$ ; \*\*  $P < 0.001$ .

Raw probability levels are given; however, all noted coefficients remained significant after following the procedure for controlling false discovery rate.

TABLE III. Results of principle component analysis on all position choice of *Oncorhynchus mykiss* fry and activity variables, with orthogonal rotation applied to the first two principal components. Values in bold face indicate interpretable loading values

	Rotated components	
	1	2
Eigenvalue	2.492	1.953
% variance explained	24.985	12.062
Total % variance explained		37.047
Component loading values		
Pre-handling position	<b>0.647</b>	<b>0.335</b>
Post-handling position	<b>0.459</b>	<b>0.418</b>
Pre-predator position	<b>0.726</b>	-0.101
Post-predator position	<b>0.662</b>	0.230
Pre-conspecific position	<b>0.684</b>	-0.114
Post-conspecific position	<b>0.499</b>	0.102
Pre-handling activity	0.033	<b>0.469</b>
Post-handling activity	0.123	<b>0.549</b>
Pre-predator activity	<b>0.374</b>	0.232
Post-predator activity	0.099	<b>0.572</b>
Pre-conspecific activity	0.122	<b>0.435</b>
Post-conspecific activity	-0.020	<b>0.699</b>

positive values (three coefficients were below zero; mean  $\pm$  s.d. =  $0.14 \pm 0.10$   $r_s$ , range: -0.08 to 0.33).

There was no relationship between PC-open and fry body size ( $r_s$  for ln-transformed  $L_F$  and  $M$  of 0.04 and 0.05, respectively,  $P > 0.05$  in both cases). PC-activity was significantly correlated with fry  $M$  ( $r_s = 0.21$ ,  $P < 0.05$ ) but not with  $L_F$  ( $r_s = 0.16$ ,  $P > 0.05$ ). While both behavioural indices predicted mean feeding rates, however, fry body size (expressed as ln-transformed  $L_F$ ) did not inform feeding performance (multiple regression, d.f. = 1, PC-open and PC-activity,  $P < 0.001$ , ln-transformed  $L_F$ ,  $P > 0.05$ ).

Of the 126 individuals tested, 31 fry approached the conspecific at least once, while 95 did not approach the conspecific at all. Among the responders, the mean  $\pm$  s.d. number of approaches was  $2.8 \pm 2.7$ , and ranged from one to 13 approaches. Individuals that approached a conspecific had slightly longer  $L_F$  and higher scores for the activity index than non-responders, but the two categories were not different in their preference for open positions (nominal logistic regression, d.f. = 1; ln-transformed  $L_F$ ,  $P < 0.05$ ; PC-activity,  $P < 0.001$ ; PC-open,  $P > 0.05$ ).

## POST-OBSERVATION GROWTH AND SURVIVAL

During the first growth period (28 to 38 days), there were significant effects of rearing tank, tank section and study block on  $G_M$  and effects of tank and tank section on  $G_L$  [three-level nested ANOVA,  $M$ : tank:  $F_{1,97}$ ,  $P < 0.05$ ; section (tank):  $F_{2,97}$ ,  $P < 0.01$ ; block (Tank, section):  $F_{4,97}$ ,  $P < 0.01$ ;  $L_F$ : tank:  $F_{1,97}$ ,  $P < 0.05$ ; section (tank):  $F_{2,97}$ ,  $P < 0.05$ ; block (tank, section):  $F_{4,97}$ ,  $P > 0.05$ ]. Over the longer

growth period (72 to 89 days), there were significant effects of study block on  $G_M$  and effects of tank section and study block on  $G_L$  (three-level nested ANOVA,  $M$ : tank:  $F_{1,89}$ ,  $P > 0.05$ ; section (tank):  $F_{2,89}$ ,  $P > 0.05$ ; block (tank, section):  $F_{4,89}$ ,  $P < 0.01$ ;  $L_F$ : tank:  $F_{1,89}$ ,  $P > 0.05$ ; section (tank):  $F_{2,89}$ ,  $P < 0.05$ ; block (tank, section):  $F_{4,89}$ ,  $P < 0.05$ ). Since rearing densities and feeding rates were standardized among tanks and tank sections, effects of rearing unit on growth were probably caused by low sample size (due to tag loss) of observation fish in some tank sections or within study blocks or uncontrollable differences in environmental conditions. For further analysis, significant effects of rearing unit or study block were corrected by adjusting growth rates such that the average growth rate for each tank section or study block was equivalent to the grand mean. Specifically, mean growth rate was calculated for the entire population and for each study block, and then values of individual growth rates were adjusted by the difference between the average growth rate for the appropriate study block and the grand mean. This adjustment preserved the variation in growth rate among individuals while masking effects of rearing unit or study block.

Indices of fry position choice and activity did not predict subsequent growth rate (multiple regression,  $G_L$  28 to 38 days interval,  $n = 98$ , d.f. = 1,  $P > 0.05$  for both PC-open and PC-activity; 72 to 89 days interval,  $n = 90$ , d.f. = 1,  $P > 0.05$  for both indices). There were also no differences in  $G_L$  between individuals that approached a conspecific and those that did not ( $t$ -tests, 28 to 38 days,  $n_{\text{responders}} = 24$ ,  $n_{\text{non-responder}} = 74$ ;  $P > 0.05$ ; 72 to 89 days,  $n_{\text{responders}} = 20$ ,  $n_{\text{non-responder}} = 70$ ,  $P > 0.05$ ).

Of the 126 fry observed, three fish died of unknown causes 3–5 days after behavioural observations were complete and a fourth mortality occurred after one fish was accidentally injured during capture for VIE tagging. The three fish that died of unknown causes were not different from the majority of the fish that survived in PC-open or PC-activity indices, or in their average number of feedings during behavioural observations ( $t$ -test,  $P > 0.05$  for all tests).

## DISCUSSION

Behavioural syndromes are characterized by individual consistency in behaviour and correlated behaviours across multiple contexts or situations. In the present study, position choice and activity rates in newly emerged *O. mykiss* were significantly correlated across safe (pre-stimulus) and unsafe situations (after a handling experience and threat of predation), as well as a social context (in the presence of a conspecific). While individual differences in risk-taking behaviour have been documented previously in *O. mykiss* with older subjects (Sneddon, 2003; Wilson & Stevens, 2005), behavioural consistency in younger subjects at the outset of juvenile feeding suggests that temperament may play a role throughout a broader period of early development.

Behavioural consistency was strongest with respect to time spent in open areas (14 of 15 possible correlations across contexts were significant, compared with eight significant relationships in activity levels; Tables I and II). Interestingly, for each of the assays, plots of time spent in the open revealed a clearly bimodal distribution of position choice. For example, 45% of all subjects were observed in the open for each of the 10 point observations before the predator attack, while 35% were always observed

in sheltered positions, accounting for 80% of all the fry observed (Fig. 2). After the simulated predator attack, the distribution remained bimodal, with 38% always in open positions and 53% consistently in hiding (91% of all observations; Fig. 2). Similar distributions were evident before and after both handling stress and the introduction of a conspecific. In *O. mykiss* parr (Sneddon, 2003) as well as other taxa (Benus *et al.*, 1991), such bimodal distributions of behavioural traits represent alternative, but equally successful, coping strategies. Individuals with higher indices of time spent in the open also had higher feeding rates; thus, bimodal position choice distributions did not reflect equally viable alternative feeding strategies, but rather differences in behavioural type that influenced performance during the behavioural assays.

While correlations between multiple observations of individual behaviours were statistically significant, it is notable that the values of  $r_s$  were not all exceptionally high (statistically significant values of  $r_s$  ranged from 0.21 to 0.46; Tables I and II). These modest values of correlation coefficients raise the possibility of low biological importance represented by correlations, despite their statistical significance. Correlations of the magnitude reported by the present study, however, are not unprecedented among previous studies demonstrating behavioural syndromes (Lee & Berejikian, 2008b). It is not clear whether such correlations are likely to affect performance or fitness of individuals (Johnson & Sih, 2007). From this perspective, investigations such as the present study of the potential influence of behavioural type on individual growth rates are worthwhile in that they can help to shed light on the biological import of modest yet statistically significant results that suggest the existence of behavioural syndromes.

In addition to consistent individual variation in single behaviours, behavioural syndromes often involve suites of multiple behavioural traits that co-vary (Sih *et al.*, 2004a). In the present study, there was no strong evidence for a significant relationship between activity and position choice behaviours. In fact, the rotation of the interpretable components of the PCA revealed nearly a complete segregation of activity and position choice between the two components. Fry could avoid open areas while remaining active, for example, by swimming around the tank edges. While activity and time spent in open areas might be conceived as joint components of a boldness syndrome, Réale *et al.* (2007) have proposed that boldness describes individual reaction to risky situations, whereas activity may constitute a separate axis of personality, apart from dangerous contexts. Consistent with this general framework, time spent in open positions was correlated after presentation of the two threat stimuli (post-handling *v.* post-predator; Table II), whereas activity was not significantly related between these two risky situations. Instead, activity was more likely to be correlated across safe contexts (pre-stimuli; Table I). Therefore, individual consistency in position choice may be a more accurate reflection of behavioural type with respect to boldness rather than activity. Activity scores, in turn, were higher among fry that exhibited an aggressive response to a conspecific. Aggression rates, however, were generally low, with only one-quarter of the fry observed responding to the introduced conspecific at all. Furthermore, both activity scores and response to a conspecific were positively related to fry  $L_F$ , suggesting that these relationships among behaviours were not wholly dependent on individual temperament, but also on body size.

In summary, behavioural observations of young *O. mykiss* in the present study revealed a modest degree of individual consistency in behaviour, particularly with

respect to time spent in exposed tank areas. Individual behavioural type, however, did not involve correlations between activity and position choice. As with the moderate correlation coefficients discussed above, it is important to note here that the PCA condensing the two behaviours into single axes explained less than half of the variance in the data (37%; Table III). The fact that a larger proportion of the total variance went unexplained by the interpretable components reflects small correlation coefficients in the original matrix for the PCA that included both activity and position choice, as well as the modest yet significant correlations between observations of individual behaviours. This moderate percentage of variance explained by interpretable components of the PCA brings into question the stability of behavioural type with respect to activity and position choice behaviours in young *O. mykiss* fry, even if the two components retained from the PCA appear to reflect individual consistency in single behaviours. Instead, behavioural type may become more stable over ontogeny. Longitudinal studies on fishes (Budaev *et al.*, 1999; Bell & Stamps, 2004) and mammals (Loughry & Lazari, 1994) have demonstrated stronger individual consistency in older animals. It is possible that at a slightly older age, stronger links between activity, boldness and aggression might be observed among the subjects observed in the present study, but a second matching dataset would be necessary to examine stability or consolidation of individual behavioural type.

Beyond an assessment of boldness and activity syndromes among newly emerged *O. mykiss*, a second goal of this study was to investigate the effect of hatchery selection with respect to behavioural type in a conventional hatchery rearing system. While this study did not specifically compare the variability or average risk-taking behaviour between hatchery origin and wild *O. mykiss* fry, if breeding and rearing practices at Warm Springs Hatchery selected for a specific fry behavioural type (*e.g.* a more bold, aggressive type), lower behavioural variation than observed would have been expected, particularly given recent evidence for a genetic basis in boldness in *O. mykiss* (Azuma *et al.*, 2005). Instead, newly emerged fry in the present study were characterized by notable behavioural variation, particularly with respect to position choice (Fig. 2).

Despite this early variation in behaviour, and evidence for individual consistency in position choice and activity, there was no relationship between indices of fry behaviour and survival or early growth rates in a conventional hatchery rearing environment. Based on the positive relationship between behavioural indices and feeding rate, a logical prediction would be that individuals with high scores for PC-open and PC-activity should achieve faster growth rates during early development. Regardless, no relationship between growth rate and fry behaviour in either position choice or activity was apparent. This result could be due to unstable behavioural types among *O. mykiss* fry at the onset of the juvenile feeding stage, such that individual behavioural profiles during the 72 to 89 day growth period were different than those observed prior to the growth period. It is important to acknowledge here, however, that the rearing context in the present study was a social, high density environment, whereas fry behavioural type was assessed among solitary individuals. Behavioural expression may vary between these social contexts. A number of previous examples have demonstrated context specificity of behavioural type in fishes (Coleman & Wilson, 1998; Bell & Stamps, 2004; Magnhagen & Staffan, 2005; Wilson & Stevens, 2005; Magnhagen, 2007). Since neither the persistence of behavioural type over time or across social contexts were investigated here, context specificity or instability of

behavioural type during the first months of hatchery rearing cannot be ruled out as possible explanations for the lack of correlation between fry behavioural type and growth rate.

The lack of a relationship between measurements of indices of individual fry behaviour and growth rate, however, is consistent with recent work in Arctic char *Salvelinus alpinus* (L.) in which no difference in antipredator behaviour was observed between fast and slow-growing groups raised in a standard hatchery conditions (Laakkonen & Hirvonen, 2007). The results of the present study and from work on *S. alpinus* support the hypothesis that relaxed selection with respect to risk-taking behaviour may allow for high behavioural variability (McPhee, 2004). This is not to say, however, that hatchery conditions do not impose directional selection on other behavioural traits, not measured here, which may affect fitness in captivity and in the wild, post-release environment. Recent work in zebrafish *Danio rerio* (Hamilton) comparing behavioural syndromes between one wild and two laboratory-derived strains has shown clear differences between wild and domesticated strains, but also between domesticated strains (Moretz *et al.*, 2007). This result suggests that effects of captive environments on behavioural phenotype may be variable and are likely to be dependent on the both the strain undergoing domestication and the particularities of the context during selection.

While there is rising interest in understanding the behaviour of farmed fishes and how domestication influences population-level distributions of behavioural traits (Shumway, 1999; Huntingford, 2004; Huntingford & Adams, 2005; Salvanes & Braithwaite, 2006), further research will be needed to determine the stability of behavioural syndromes across ontogeny, their relationship with growth and survival in young salmonids, as well as how such patterns may vary across captive environments. In view of the importance of maintaining behavioural diversity in captive populations that is ecologically relevant for population persistence in post-release conditions, such research will probably aid in the success of population enhancement programmes.

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